

Homocytotropic antibodies (IgE) to *Neisseria gonorrhoeae* in the rat and a cross-reactivity of heterologous gonococcal strains

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SUMMARY Outbred Wistar rats were immunised with a single intraperitoneal injection of a mixture of 30 mg of $Al(OH)_3$ and 100 μ g of gonococcal zeolite antigen (ZA). Ten days after immunisation, ZA prepared from T_1 and T_4 colonies of *Neisseria gonorrhoeae* strain GC6 (GC6- T_1 ZA and GC6- T_4 ZA) were able to elicit a reaginic (IgE) response which declined to low levels by 35 days. There was a significant amount of histamine release from the mast cells of actively-sensitised rats on challenge with a specific gonococcal antigen. The antisera heated at a temperature of 56 °C for four hours failed to elicit a passive cutaneous anaphylaxis (PCA) reaction, indicating a lack of IgG antibody. In addition antisera to GC6- T_1 ZA gave a positive PCA reaction with GC6- T_4 ZA and ZA prepared from T_1 colonies of six heterologous gonococcal strains suggesting that these strains of *N. gonorrhoeae* share common antigenic determinants.

Introduction

Gonorrhoea remains one of the world's most commonly reported communicable diseases (Kiraly and Causse, 1975). A factor responsible for the current epidemic is the difficulty in detecting asymptomatic carriers of gonorrhoea (Pariser and Marino, 1970; Pariser, 1972). Thus, a reliable serodiagnostic test to detect asymptomatic carriers of gonorrhoea would greatly help to control the disease.

Several serological tests—such as, complement fixation, flocculation, microprecipitin, and passive haemagglutination—have been used with limited success for the serodiagnosis of gonorrhoea (Kellogg and Balows, 1976). Also, some studies have been carried out on the response of specific immunoglobulins IgA, IgG, IgM, and secretory IgA to gonococcal infection employing whole cells or purified pili as antigens (Cohen *et al.*, 1969; Buchanan *et al.*, 1973; O'Reilly *et al.*, 1976).

Of particular interest is a report that the mean serum IgE level was higher in a group of patients with gonorrhoea compared with a group of normal individuals (Green *et al.*, 1976). However, little is known about reaginic (IgE) antibody response to antigenic stimulus of *Neisseria gonorrhoeae*. It has been shown that IgE antibodies are produced in

response to infections in rats by parasites (Perrudet-Badoux *et al.*, 1976). Also, IgE antibodies are formed in mice immunised with water soluble antigens isolated from certain Gram-negative bacteria (Danneman and Michael, 1976).

The current study was designed to develop an animal model for the production of IgE antibodies to gonococcal antigens. This model would be useful for the monitoring of purified gonococcal antigens which are capable of stimulating IgE antibody response. Such antigens might be employed for the serodiagnosis of gonorrhoea based on IgE antibody formation to infection with *N. gonorrhoeae* in humans.

Material and methods

BACTERIA

The source of *N. gonorrhoeae* strains GC6, 188, IN31, and G9 has been described previously (Perry *et al.*, 1975). Other strains (LCDC No. GC-15, 74123 and 74002) were obtained from the Regional Public Health Laboratory, Bell's Corner, Ontario. The strains were maintained as colony type 1 (T_1) or 4 (T_4) by daily passage on GC medium (Kellogg *et al.*, 1963).

PREPARATION OF ZEOLITE ANTIGENS

One gram (wet weight) of viable gonococci was placed in a mortar in an ice-bath and 2 g of zeolite (type 4A, received as a gift from Union carbide,

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Linde Division, New York, NY) was added slowly while grinding with a pestle for three minutes. Grinding was continued for another three minutes while 100 ml of cold distilled water was added to the mortar. The suspension was centrifuged at 3000 *g* to remove intact gonococci and zeolite particles. The supernatant was centrifuged at 40 000 *g* for one hour at a temperature of 4°C to remove particulate material. The supernatant was designated as zeolite antigen (ZA).

PROTEIN CONCENTRATIONS

The content of protein in each preparation was determined by the method of Lowry *et al.* (1951). Bovine albumin (Fraction V from bovine plasma) obtained from Armour Pharmaceutical Company, Chicago, Illinois was used as a standard.

IMMUNISATION OF RATS

Two groups, each consisting of 10, male outbred Wistar rats weighing 225-250 g (Woodlyn Farms, Guelph, Ontario) were immunised with a single intraperitoneal injection of 1.0 ml of mixture containing 100 µg of ZA prepared from *N. gonorrhoeae* strain GC6, colony type 1 or 4 (GC6-T₁ ZA or GC6-T₄ ZA) precipitated with 30 mg of aluminum hydroxide gel (Al (OH)₃). The animals were given halothane anaesthesia and bled by cardiac puncture at 10, 22, and 35 days after immunisation. Not more than 3 ml were withdrawn if the rats were to be kept. After allowing the blood to clot at 25°C for between one and two hours followed by standing overnight at 4°C, the serum was separated by centrifugation and stored at -70°C until used in passive cutaneous anaphylaxis (PCA) reactions.

ANTIBODY DETERMINATIONS

The level of circulating reagins (IgE) was estimated by PCA test (Ovary, 1964) with a sensitisation period of 48 hours. Thus 0.1 ml quantities of saline dilutions of test serum were injected intradermally into the skin of recipient rats (outbred Wistar) and each injection was duplicated on different animals. The animals were injected intravenously 48 hours later with either 0.6 ml (1.6 mg/ml protein) of GC6-T₁ ZA or 0.75 ml (1.35 mg/ml protein) of GC6-T₄ ZA mixed with 0.5 ml of 2% Evans's blue dye. The skin reactions were examined after 30 minutes. The antibody titres were determined in triplicate and are expressed as the reciprocal of the highest dilution giving a diameter greater than 5 mm in the blueing reaction.

An attempt was also made to detect heat-stable (IgGa) homocytotropic antibody by performing the four-hour PCA tests with sera heated at 56°C for four hours.

HISTAMINE RELEASE FROM MAST CELLS OF ACTIVELY SENSITISED RATS

Two rats from each group were selected randomly on days 10, 22, and 35 after immunisation. The cells were harvested from the rats as reported earlier (Vijayanagar *et al.*, 1974). A modified technique of Evans and Thomson (1972) was used to determine the ability of the mast cells from actively sensitised rats to release histamine on challenge with homologous zeolite antigens either GC6-T₁ ZA or GC6-T₄ ZA. The challenging dose of the antigens used in histamine release ranged from 1-200 µg.

Results and discussion

REAGINIC ANTIBODY RESPONSE

Table 1 shows the mean homocytotropic antibody titre in the sera of rats immunised with gonococcal antigens. It is evident that reaginic (IgE) antibodies of maximum response were present in the sera of the rats 10 days after immunisation with GC6-T₁ ZA and GC6-T₄ ZA. These reagins had declined by day 22 (maximum PCA titre, 16) and were hardly detectable on day 35 (PCA titre, 0-1). Antiserum to GC6-T₁ ZA gave a significant PCA titre when challenged with GC6-T₄ ZA and vice versa.

Table 1 Mean* homocytotropic (IgE) antibody titres in rats immunised with gonococcal (zeolite) antigens

Immunising antigen	No. of rats positive†			Reciprocal PCA titres on challenge with								
				GC6-T ₁ ZA			GC6-T ₄ ZA					
	Days after immunisation											
	10	22	35	10	22	35	10	22	35	10	22	35
GC6-T ₁ ZA	10/10	8/8	1/6	64	4	2	128	16	1			
GC6-T ₄ ZA	10/10	6/8	0/6	32	8	0	128	16	0			

*In each group, positive sera were pooled.

†Undiluted sera of the rats were tested by PCA reaction using GC6-T₁ ZA as a challenging antigen.

HISTAMINE RELEASE FROM MAST CELLS OF ACTIVELY SENSITISED RATS

Mast cells from actively sensitised rats were challenged with homologous ZA to determine if there was cellbound IgE specific to the gonococcal zeolite antigens. Table 2 shows that 10 days after immunisation, there was 60-84% histamine release which declined to 23-47% after 22 days. By day 35 there was still an appreciable amount of histamine release from mast cells of rats immunised with GC6-T₄ ZA. However, there was no histamine release in rats immunised with GC6-T₁ ZA. Since the rats were selected randomly for histamine release and the PCA titres were done on pooled antisera, it was not

Table 2 *Per cent of histamine release from actively sensitised rat peritoneal mast cells on challenge with zeolite antigens prepared from N. gonorrhoeae strain GC6*

Immunising antigen	Actively sensitised rat peritoneal mast cells challenged with	Per cent of histamine release from actively sensitised rat peritoneal mast cells challenged with ZA		
		Days after immunisation		
		10	22	35
GC6-T ₁ ZA	GC6-T ₁ ZA	84	23	0
GC6-T ₄ ZA	GC6-T ₄ ZA	60	47	36

possible to correlate the amount of histamine release with the corresponding PCA titre. However, it is noteworthy that on day 35 after immunisation, although no PCA reaction could be detected in the sera of animals immunised with GC6-T₄ ZA (Table 1), there was a considerable amount (36%) of histamine release from the mast cells of these rats on challenge with GC6-T₄ ZA. These results suggest that cellbound IgE does not always parallel the amount of circulating antigen specific IgE antibodies. These observations are in agreement with those of Yamamoto *et al.* (1974).

In addition, it is important to point out that before a challenge with the specific gonococcal antigen, a large number of actively sensitised mast cells were degranulated. If this is true, the leucocytes of patients infected with gonorrhoea should be studied.

CROSS-REACTIVITY OF GC6-T₁ ZA ANTISERA WITH HETEROLOGOUS GONOCOCCAL STRAINS

In order to examine the cross-reactivity of GC6-T₁ ZA with zeolite antigens prepared from other strains of *N. gonorrhoeae*, antisera obtained from rats 10 days after immunisation, were challenged with ZA prepared from T₁ colonies of six heterologous gonococcal strains. The results in Table 3 indicate that GC6 T₁ ZA antiserum contained IgE antibody which cross-reacted with ZA from all six hetero-

Table 3 *Cross-reactivity of GC6-T₁ ZA antiserum with ZA obtained from T₁ colonies of six different strains of N. gonorrhoeae*

Challenging zeolite antigen	Reciprocal PCA titre*
GC6	64
G9	64
IN31	128
74123	64
188	64
GC15	128
74002	128

*Determined with sera obtained from rats 10 days after immunisation with GC6-T₁ ZA.

logous strains of *N. gonorrhoeae*. The antisera heated at 56°C for four hours failed to elicit a four-hour PCA reaction, indicating a lack of IgGa antibody. These results suggest that these strains have common antigenic determinants and support previous studies which indicate that one or more common proteins to several gonococcal strains are present in the ZA (Wallace *et al.*, 1977). Studies are currently under way to isolate the specific antigen(s) which are responsible for the production of IgE in rats and are common among the various gonococcal strains. Such antigens may be useful in the development of a serodiagnostic test for gonorrhoea based on the detection of IgE antibodies produced in response to gonococcal infection.

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